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## Critical Windows of Exposure for Children's Health: Cancer in Human Epidemiological Studies and Neoplasms in Experimental Animal Models

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### Abstract

In humans, cancer may be caused by genetics and environmental exposures; however, in the majority of instances the identification of the critical time window of exposure is problematic. The evidence for exposures occurring during the preconceptional period that have an association with childhood or adulthood cancers is equivocal. Agents definitely related to cancer in children, and adulthood if exposure occurs *in utero*, include: maternal exposure to ionizing radiation during pregnancy and childhood leukemia and certain other cancers, and maternal use of diethylstilbestrol during pregnancy and clear-cell adenocarcinoma of the vagina of their daughters. The list of environmental exposures that occur during the perinatal/postnatal period with potential to increase the risk of cancer is lengthening, but evidence available to date is inconsistent and inconclusive. In animal models, preconceptional carcinogenesis has been demonstrated for a variety of types of radiation and chemicals, with demonstrated sensitivity for all stages from fetal gonocytes to postmeiotic germ cells. Transplacental and neonatal carcinogenesis show marked ontogenetic stage specificity in some cases. Mechanistic factors include the number of cells at risk, the rate of cell division, the development of differentiated characteristics including the ability to activate and detoxify carcinogens, the presence of stem cells, and possibly others. Usefulness for human risk estimation would be strengthened by the study of these factors in more than one species, and by a focus on specific human risk issues. **Key words:** cancer, chemical carcinogens, childhood, exposure, fetus, *in utero*, ionizing radiation, neonatal, postnatal, preconception. -- *Environ Health Perspect* 108(suppl 3):573-594 (2000).

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Cancer (as well as other human diseases) may be caused by genetic and environmental factors, the relative contribution of each to disease etiology varying from one malignancy to another. In developed countries, cancer is principally a disease of the elderly, with the overall incidence rising steadily with increasing age (1). Cancer in children younger than 15 years of age is rare, accounting for < 1% of malignancies diagnosed each year in developed countries (1,2). Furthermore, although there is some degree of overlap, the types of cancer that occur in young people tend to differ histopathologically, biologically, and clinically from those that occur at older ages (3).

The majority of cancers diagnosed in children and adolescents are aggressively invasive and grow rapidly. Typically, they are more receptive to chemotherapy than those that occur at older ages, and the last 20 years have seen significant increases in survival over a wide range of diagnostic groups (1,3-5). Unfortunately, these marked improvements in cancer treatment have not been matched by similar insights into cancer etiology, and the cause or causes of the majority of malignancies in children and young adults remain unknown (2,3,6). In fact, with few exceptions, there is little evidence to link the majority of childhood and adolescent cancers with the well-known carcinogens implicated in adult-onset disease. That having been said, exposures in childhood are recognized determinants of certain cancers; for example, early childhood exposure to hepatitis B virus (HBV) is critical for the development of hepatocellular carcinoma (7,8) and there is much circumstantial evidence to support the suggestion that exposure to infectious agents in the first few years of life could be an important risk factor for the development of acute lymphoblastic leukemia (9).

Exposures *in utero* have long been thought to be important determinants of certain cancers occurring in children and young adults and in recent years attention has focused on the possible etiological roles of a variety of factors acting at this critical time in human development. Epidemiological evidence that prenatal exposures are involved in the development of childhood malignancy was first provided by the Oxford Survey of Childhood Cancers over 40 years ago when an association between diagnostic radiography of mothers during pregnancy was related to the subsequent development of leukemia and other cancers in their children (10,11). Although this association was initially greeted with skepticism, it is now generally accepted that the fetus and young child may be more susceptible to the effects of ionizing radiation than the adult, with recent concern revolving mainly around the importance of

dose and gestational age at the time of exposure (12,13). Interest in the potential carcinogenic effects of *in utero* exposures was rekindled in 1971 when Herbst et al. (14) reported an association between the development of clear-cell adenocarcinoma of the vagina in young women and their mothers' use of diethylstilbestrol (DES) during pregnancy. Recently, although no candidate exposures were identified, Ford et al. (15) provided molecular evidence that rearrangements at the genetic locus 11q23, seen in the majority of infant leukemias, could originate *in utero* and, in a further report, they suggested that T-lineage malignancies in older children could also be initiated *in utero* (16).

Animal studies have confirmed that a variety of types of radiation and chemical carcinogens given either preconceptionally, *in utero*, or directly to the neonate can result in an increased incidence of neoplasms in the offspring (17,18). However, there is a clear lack of comparable human data on this topic. At present, the only generally accepted carcinogenic *in utero* exposures in humans are to ionizing radiation and DES, and the suggestion that parental preconceptional exposures could potentially influence the risk of cancer in their offspring is controversial. Nonetheless, the list of *in utero* and preconceptional factors suggested as possible risk factors for human cancer is ever-lengthening: the most frequently discussed agents are low-dose ionizing radiation, hormones (endogenous and exogenous), infections (specific and nonspecific), and a variety of chemicals and drugs (9,19-27).

This review considers the evidence that critical windows of exposure exist for human cancers and presents the relevant data from animal models, along with a discussion of mechanisms. We also discuss the knowledge gaps in both the human and animal data and consider some of the options for future research.

## Humans

### Exposure Periods

Figure 1 presents a simplified schematic framework for considering cancer etiology in relation to the timing of exposure with an example of each pathway. In humans, identifiable familial hereditary cancer syndromes are rare, probably accounting for < 1% of cancers overall (28,29). For other malignancies, the investigation of critical time windows of exposure could span back to the conception of an individual's parents (Figure 1). The parental *in utero* time period may be more relevant for mothers than for fathers because oocytes begin their maturation during gestation and no new ones are formed after birth. By contrast, in terms of preconceptional transmission, exposures occurring during an individual's father's life may be more important than exposures occurring during their mother's life. This is because spermatogenesis continues from puberty to old age and hence there is more opportunity for preconceptional mutant gene accumulation in men than women (30).

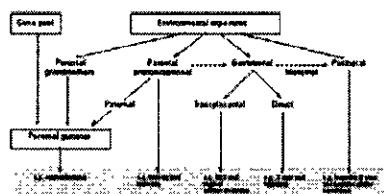


Figure 1. Schematic framework for considering cancer etiology.

### Transgenerational/Preconceptional/Periconceptional Exposure

Animal experiments have shown that the exposure of germ cells to carcinogens and mutagens leads to an excess of tumors in offspring (17,31). Despite substantive animal evidence, there are presently no generally accepted direct links between the two in humans. However, there are certain nonfamilial recognizable genetic conditions (the etiologies of many of which are unclear) that predispose toward certain cancers. Although some are clear defects (e.g., trisomy 21), others are not (e.g., ethnicity and sex). The effect of sex deserves particular attention because in most populations males are significantly more likely to be diagnosed with cancer than females. Further, sex selection occurs in certain animal species (32) and there is epidemiological evidence that environmental risk factors may alter the probability of having a child of one sex or the other. The most frequently suggested exposures relate to paternal occupation (33-37).

The association between childhood cancer and sex is illustrated in Tables 1 and 2, where national data are presented for England and Wales for 1981-1990 and for the U.S. Surveillance, Epidemiology, and End Results cancer registry for 1983-1992 (38). In human communities, male births generally account for 51-53% of all births, yielding a sex ratio of approximately 1.05. During the first year of life, although the majority of cancers are just as likely to be diagnosed in girls as in boys, a characteristic male predominance begins to emerge with increasing age (Tables 1 and 2). These sex differences are particularly noticeable for the more common forms of childhood cancer: the overall male-to-female ratio for leukemia and tumors of the central nervous system, each of which account for around one-fifth of all childhood cancers, is approximately 1.2. However, the largest differences are seen for the lymphomas (Hodgkin and non-Hodgkin), where males are approximately twice as likely to contract the disease.

Table 1. Number of cases (N) and sex ratio (male/female) by age for England and Wales, 1981-1990.

Cancer	Total (N)	Sex ratio (M/F)	0-4	5-9	10-14	15-19
Leukemia	3,228/2,123	0.68	1.2	1.2	1.4	1.2
Lymphoma	2,088/1,048	0.7	1.2	1.3	1.6	1.3
Brain and spinal cord	1,130/1,048	0.96	1.3	1.3	2.1	2.0
Soft tissue	499/494	1.01	1.3	1.3	1.3	1.3
Other	1,048/1,048	1.0	1.3	1.3	1.3	1.3
Brain and spinal cord	2,348/2,123	1.1	1.3	1.3	1.3	1.3
Soft tissue	2,348/2,123	1.1	1.3	1.3	1.3	1.3
Other	2,348/2,123	1.1	1.3	1.3	1.3	1.3

Subsistence exposure	298 (5)	1.5	1.1	1.1	1.1	1.2
Genetic cell and parental	307 (4)	2.6	1.2	0.6	0.6	0.9
Genetics	381 (2)	-	0.9	1.0	0.8	0.8
Total	1,282 (100)	1.1	1.2	1.2	1.1	1.2

95% confidence interval (CI)

Table 2. Relative risk of cancer (95% confidence interval) by exposure to pesticides in the home, by age at diagnosis, and by cancer site.											
Cancer site	Age at diagnosis	Relative risk	95% CI	Relative risk	95% CI	Relative risk	95% CI	Relative risk	95% CI	Relative risk	95% CI
Brain	<15	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Brain	15-64	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Brain	≥65	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Leukemia	<15	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Leukemia	15-64	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Leukemia	≥65	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Neuroblastoma	<15	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Neuroblastoma	15-64	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Neuroblastoma	≥65	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Wilms' tumor	<15	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Wilms' tumor	15-64	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8
Wilms' tumor	≥65	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8	1.0	0.5-1.8

### Associations with Inherited Genetic Disorders

Recognized genetic syndromes and familial aggregation account for a relatively small proportion of total cancers--probably no more than 5%. This proportion varies with cancer type and age at diagnosis.

**Retinoblastoma.** Retinoblastoma, which is a rare cancer accounting for approximately 2% of all malignancies diagnosed in children younger than 15, has become a paradigm for considering the etiological role of genetic factors in cancer epidemiology. In 1971, Knudson (39) proposed a two-mutation hypothesis to explain the occurrence of retinoblastoma in both hereditary and sporadic forms with differing frequencies of bilaterality. Knudson suggested that in hereditary cases the first mutation occurred in a germinal cell and the second in a somatic cell, whereas in nonhereditary cases both mutations occurred postzygotically in the same somatic cell line. Hereditary cases were further subdivided into familial (accounting for approximately one-quarter of all hereditary cases and one-eighth of all cases) and nonfamilial (accounting for approximately three-quarters of all hereditary cases and three-eighths of all cases). Knudson's model has since been confirmed using molecular techniques, with heritable cases characterized by a constitutional 13q deletion (40,41).

With respect to etiology, the origins of nonfamilial retinoblastoma are unclear. The majority of new germline mutations (sporadic heritable) appear to be paternally derived, but no consistent associations with paternal exposures have yet been demonstrated (42-44). In the same study, Bunin et al. (42) reported an association between sporadic heritable retinoblastoma and self-reported maternal X ray of the lower abdomen or pelvis.

**Wilms' tumor.** Wilms' tumor is an embryonal tumor, accounting for approximately 90% of all kidney cancers diagnosed in children younger than 15 years of age (45,46). It is the fourth most common childhood cancer, accounting for approximately 6% of all cancers diagnosed before 15 years of age (46), and it occurs with equal frequency in boys and girls (Tables 1 and 2). Wilms' tumor is extremely rare in those over the age of 15, although it has been seen in adults (47).

Familial Wilms' tumor accounts for < 1% of Wilms' tumor cases; approximately 20% of familial cases are bilateral compared to 3% of sporadic cases (48). Because of similarities between Wilms' tumor and retinoblastoma, Knudson and Strong (49) initially proposed a similar two-hit mutational model to explain its occurrence in both hereditary and sporadic forms with differing frequencies of bilaterality. Subsequent studies revealed, however, that the pathogenesis of Wilms' tumor was far more complex. Further work led to the identification and cloning of the Wilms' tumor-suppressor gene (*WT1*) located at chromosome 11p13 (50). A second suppressor gene has been suggested (*WT2*); this gene is located on chromosome 11p15 (51). Associations with certain congenital anomalies have been described, including WAGR syndrome (Wilms' tumor with congenital aniridia, genitourinary abnormalities, and mental retardation), Beckwith-Wiedemann syndrome, Perlman syndrome, Denys-Drash syndrome, and hemihypertrophy (52).

As with retinoblastoma, *de novo* Wilms' tumors appear to be largely paternally derived (53). Inherited mutations of *WT1* have been demonstrated in cases of Wilms' tumor; in some instances the mutation came from the father, whereas in other cases the mutation arose during paternal gametogenesis (54,55). Genomic imprinting has been proposed as another mechanism involved in the pathogenesis of Wilms' tumor. Genomic imprinting is defined as a "gamete specific modification of a gene resulting in differential expression in somatic cells" (56). Genomic imprinting of a transforming gene on the maternally derived chromosome 11 has been suggested as an alternative explanation for preferential maternal allele loss.

With respect to etiology, several studies have demonstrated an association between childhood Wilms' tumor and father's occupational exposure to pesticides or employment in the agricultural industry (36,57-59).

**Other cancers and genetic disorders.** Associations with specific congenital anomalies have been described for a number of cancers other than Wilms' tumor. Examples of the four types of genetic disorders and the cancers with which they have been associated are given below:

- Aneuploidy: a) trisomy 21 (Down syndrome) and acute leukemia (60-70). [The relative risk of developing acute leukemia among children with Down syndrome is high--approximately 30-fold (6)] and b) monosomy X (Turner syndrome) and neuroblastoma (71).
- Point mutations: mutations in the *NF-1* gene (neurofibromatosis) and tumors of the central nervous system (72-74), rhabdomyosarcoma (75), and leukemia (72,76,77).
- Chromosomal rearrangements: duplication or unbalanced translocation of chromosome breakpoint 11p15 (Beckwith-Wiedemann syndrome) and Wilms' tumor (52).
- Chromosomal instability syndrome: fragile chromosome site at 9q22 (Fanconi anemia) and acute myeloid leukemia (78).

With respect to the origin and timing of potentially hazardous exposures (Figure 1), aneuploidy predominately arises from maternal errors of segregation at the first meiosis (79,80), whereas point mutations and chromosomal rearrangements tend to result more commonly from paternal errors (79). The origin of chromosomal instability syndromes is unclear.

**Associations with specific exposures.** The suggestion that preconceptional exposures of parents' germ cells could influence cancer risk in their offspring is controversial. Although there is more literature emerging on the potential effects of parental lifestyle factors such as smoking, diet, and alcohol consumption (81-83), the main areas of interest to date have been in the children of individuals given chemotherapy and those exposed to unusual levels of ionizing radiation. With respect to chemotherapy, the marked improvements in survival among children and young adults treated for cancer have given rise to concern about possible germ-cell damage, but, at present, the data are sparse (84-86). With respect to ionizing radiation, particular controversy surrounds the suggestion that germ-cell damage caused by low doses of external ionizing radiation might increase the risk of cancer and other genetic diseases in the progeny of those exposed. The findings for paternal occupational exposure, which is one of the few areas where good individual dose data are available, are reviewed below.

**Ionizing radiation.** The hypothesis that occupational preconceptional exposure of fathers to external sources of ionizing radiation increases the risk of leukemia in their offspring derives mainly from the case-control study conducted by Gardner et al. (87). In 1990, they wrote:

The main finding of this study is that the recorded external dose of whole body ionising radiation to fathers during their employment at Sellafield is associated with the development of leukaemia in their children.... The results suggest highest risks in those with highest accumulated ionising radiation doses before conception, either over their total duration of exposure or during the preceding six months.

These statements were based on two results in their study. Compared to the offspring of fathers with no record of monitoring for external sources of ionizing radiation at Sellafield, those whose fathers had a lifetime cumulative dose of 100 mSv or more before their child's conception were estimated as 8.4 [95% confidence interval (CI), 1.4-52.0] times more likely to develop leukemia. The four case fathers with a cumulative preconceptional dose of  $\geq 100$  mSv received 10 mSv of this dose in the 6 months before their child's conception, yielding a relative risk of 6.8 (CI, 1.5-31.9) (88).

Results from four other case-control studies (89-92) and one cohort study [Nuclear Industry Family Studies (NIFS) (27)] that examined paternal preconceptional dosimetry data are compared with those of Gardner et al. (87,88) in Tables 3 and 4. As with the West Cumbrian investigation in the United Kingdom, the studies in Caithness [which contains the Atomic Energy Authority (AEA) Dounreay plant] (89), West Berkshire [which contains the Atomic Weapons Establishment (AWE) plants at Aldermaston and Burghfield] (91), and five regions in Ontario containing a nuclear facility (90) were specifically targeted at leukemia and non-Hodgkin lymphoma cases in the vicinity of nuclear plants. The record-linkage study aimed at national coverage by using birth certificate identifiers to link U.K. registration data on all childhood cancers to data on workers included on the National Registry of Radiation Workers (92).

Table 3. Relative risk of leukemia and non-Hodgkin lymphoma in children of fathers with recorded external doses of ionizing radiation before conception. Data are from Gardner et al. (87) and other studies.

Study	Exposure Category	Relative Risk (95% CI)
Gardner et al. (87)	< 100 mSv	1.0
	$\geq 100$ mSv	8.4 (1.4-52.0)
Caithness (89)	< 100 mSv	1.0
	$\geq 100$ mSv	1.8 (1.1-3.0)
West Berkshire (91)	< 100 mSv	1.0
	$\geq 100$ mSv	1.8 (1.1-3.0)
Ontario (90)	< 100 mSv	1.0
	$\geq 100$ mSv	1.8 (1.1-3.0)
NIFS (27)	< 100 mSv	1.0
	$\geq 100$ mSv	1.8 (1.1-3.0)

Table 4. Relative risk of leukemia and non-Hodgkin lymphoma in children of fathers with recorded external doses of ionizing radiation before conception. Data are from Gardner et al. (87) and other studies.

Study	Exposure Category	Relative Risk (95% CI)
Gardner et al. (87)	< 100 mSv	1.0
	$\geq 100$ mSv	8.4 (1.4-52.0)
Caithness (89)	< 100 mSv	1.0
	$\geq 100$ mSv	1.8 (1.1-3.0)
West Berkshire (91)	< 100 mSv	1.0
	$\geq 100$ mSv	1.8 (1.1-3.0)
Ontario (90)	< 100 mSv	1.0
	$\geq 100$ mSv	1.8 (1.1-3.0)
NIFS (27)	< 100 mSv	1.0
	$\geq 100$ mSv	1.8 (1.1-3.0)

Lack of human data is one of the main messages that comes across from Tables 3 and 4. In addition, the U.K. studies are not independent: NIFS (27) and the record-linkage study (92) have the potential for overlap with the case-control studies conducted in West Cumbria (87), Caithness (89), and West Berkshire (91). For fathers monitored for radiation exposure and for those exposed to  $\geq 100$  mSv before their child's conception, the effect of this overlap is small for all but the West Cumbrian study. More importantly, perhaps, the NIFS and the record-linkage studies have the potential for overlap with each other: approximately half of those listed on the National Registry for Radiation Workers that were defined as eligible for the record-linkage study were employed at one time by the AWE, AEA, or British Nuclear Fuels Limited (92). Indeed, the relative risk estimates for leukemia and non-Hodgkin lymphoma in offspring, in relation to paternal monitoring for exposure to ionizing radiation before their child's conception, are virtually the same in NIFS and the record-linkage study: both studies estimated that the children of monitored workers had an approximate 80% increased risk compared to the children of nonmonitored workers. Despite this similarity, there is little evidence in the record-linkage study of any increased risk in the 100-mSv dose categories. Indeed, in the record-linkage study, the significantly raised risk of 1.8 (CI, 1.1-3.0) for leukemia and non-Hodgkin lymphoma among children of monitored fathers has its origins in an 8-fold elevation in risk (CI, 1.2-4, based on six cases) among those with dose levels below detection (92). Such low doses were rare in NIFS: only 2% of liveborn children were conceived by a father who had a cumulative whole-body dose of  $< 0.1$  mSv (93).

Data of the type shown in Tables 3 and 4 are sparse and cannot be broken down into nonoverlapping exposure periods. In all of the studies, fathers exposed to high doses of external ionizing radiation immediately before conception were also more likely to be exposed to high doses before that time. Similarly, fathers exposed before their child was conceived were often exposed throughout

their child's life. In addition to being unable to isolate relevant time windows of exposure, the nature of the hazardous exposure—if it exists—behind the associations depicted in Tables 3 and 4 remains unknown.

**In Utero Exposure**

Unlike the situation for preconceptional exposures, there is good evidence that exposure of the human fetus to certain potentially harmful agents can increase the risk of cancer during childhood and possibly during early adulthood (11,14-16,67,68,94-96). Nonetheless, although numerous potentially harmful agents are suspected—including infections, drugs, and maternal lifestyle characteristics [reviewed by Little (6)]—the only two generally accepted carcinogenic *in utero* exposures are ionizing radiation and DES: the former acting directly on the fetus and the latter acting via the placenta (Figure 1).

The strong associations for DES have led researchers to postulate *in utero* effects for other endogenous and exogenous hormones, particularly for cancers with a suspected hormonal component to their etiology—such as breast and testicular cancers (97-101). Further, since the birth of the first test-tube baby in 1978 there has been concern about the health of offspring resulting from assisted reproductive technology (ART). Multiple pregnancies often result from ART, which is one of the main determinants of the health of the child at birth (102-104). The importance of follow-up studies of these children to assess adverse health outcomes diagnosed after birth, even in adulthood, has been recognized, but few comprehensive and powerful epidemiological studies have been done. Two case reports have highlighted possible increases in cancer incidence in children born as a result of *in vitro* fertilization (105,106), raising concerns about the role of prenatal exposure (before and after conception) to high levels of estrogen and related compounds used for ovarian stimulation. To date, there are limited epidemiological data on this topic; a study of U.K. births after ART failed to find an excess incidence of childhood cancer (24), but, as noted by the authors, the study was too small to be able to detect a reasonable excess, even if it existed.

With respect to mechanisms and the timing of exposure, it is thought that the carcinogenic effects of both ionizing radiation and DES may be mediated via teratogenesis (107,108). This has been documented for DES, which causes various genital tract abnormalities in males as well as in females (107,109). In addition, it has been suggested that the exposure of pregnant women to substances that inhibit the function of the topoisomerase II enzymes could be related to the development of acute leukemia in their offspring (9).

**Perinatal/Postnatal Exposures**

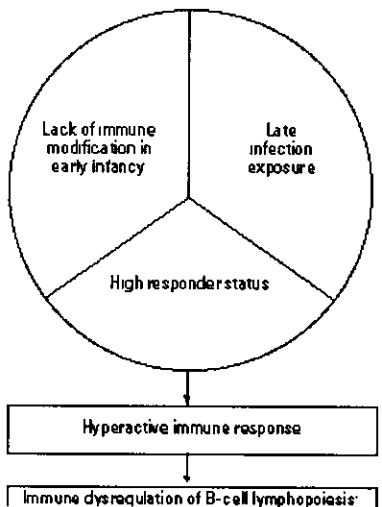
The list of environmental exposures that increase cancer risk is exceedingly long and, for the most part, outside the scope of this review. We discuss only those agents where childhood exposure is either known, or suspected, to be critical to cancer development.

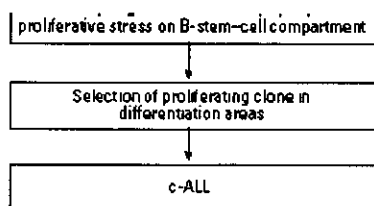
**Vitamin K.** The efficacy of intramuscular (IM) vitamin K prophylaxis in preventing both hemorrhagic diseases in neonates and late-onset bleeding disorders in breast-fed babies is well established (110). The prophylactic administration of vitamin K to neonates became a controversial topic when Golding et al. (111) reported in 1992 that children who received it by the IM route were almost 3 times more likely to develop leukemia than children who received it orally or not at all. Although subsequent studies failed to confirm these findings (95,112-119), inconsistencies in their results left lingering doubts about the safety of administering parenteral vitamin K (120). The main results of the studies conducted to date are presented in Table 5. After neonatal administration of IM vitamin K, the risk estimates ranged from 0.47 to 2.65 for all childhood leukemias. The largest risk estimate arose from the relatively small, hypothesis-generating study conducted in Bristol, United Kingdom (111).

Table 5. Estimated relative risks for the association between neonatal administration of IM vitamin K and childhood cancer.

Reference	Country	Study design	IM n/N	OR 95% CI	Age range
Golding et al. (111)	England	Cohort	280/1,336	2.65 1.38-5.08	0-14 years
Waldron et al. (112)	England	Cohort	192/1,336	0.50 0.20-1.16	1 month-14 years
Stallard et al. (113)	United States	Cohort	64/1,336	0.47 0.14-1.58	0-7 years
Olson et al. (114)	Sweden	Cohort	152/1,336	0.88 0.38-2.08	1-12 years
Frederick et al. (115)	England	Cohort	129/1,336	0.76 0.29-2.00	0-14 years
van Buren et al. (116)	Netherlands	Cohort	108/1,336	0.68 0.28-1.68	1 month-14 years
McGee et al. (117)	Scotland	Cohort	129/1,336	0.88 0.38-2.08	0-14 years
Parker et al. (118)	England	Cohort	129/1,336	0.76 0.29-2.00	0-14 years
Parker et al. (119)	England	Cohort	129/1,336	0.76 0.29-2.00	0-14 years
Frederick et al. (120)	England and Wales	Cohort	152/1,336	0.88 0.38-2.08	1-12 years
Frederick et al. (121)	England and Wales	Cohort	152/1,336	0.88 0.38-2.08	1-12 years

OR, relative risk.  
95% CI, 95% confidence interval.





**Figure 2.** An immunological model for the etiology of childhood common acute lymphoblastic leukemia (c-ALL). Adapted from Greaves (9).

**Infections.** Specific infectious/parasitic agents are now recognized as major causes of certain cancers, including various types of papilloma viruses in cervical cancer; hepatitis B and C in hepatocellular carcinoma; HTLV-1 in T-cell leukemia/lymphoma; Epstein-Barr virus in Burkitt lymphoma; *Helicobacter pylori* in gastric cancer; HHV-8 in Kaposi sarcoma; and *Schistosoma haematobium* in bladder cancer [reviewed by Newton et al. (121)]. Overall, approximately 15% of all human malignancies can be attributed to viral, bacterial, or helminth infections.

Age at first exposure--when chronic or persistent infection begins--is critical for the development of many of these malignancies. For example, children infected with HBV perinatally by their mothers have an 85-100% chance of becoming chronic carriers, compared to a 20-30% chance if they are infected between the ages of 1 week and 5 years. Although HBV persistence is a major risk factor for hepatocellular carcinoma (accounting for > 50% of the disease), the mechanisms by which persistence is established and hepatocellular carcinoma ensues is still the subject of much current research (8). In contrast to viruses such as HBV, many of which are directly oncogenic, many bacteria and helminthes act indirectly by causing tissue damage. Nonetheless, as with HBV, the establishment of a persistent infection in childhood has been suggested to be associated with the largest increase in disease risk: childhood exposure to *Helicobacter pylori*, for example, is thought to be more important for gastric cancer development than exposure at older ages (122).

In addition to the classic associations with persistent infections, there is considerable speculation about the role that infectious agents may play in the etiology of leukemia [reviewed by Little (6), Greaves (9), and Kinlen (23)]. The two main hypotheses, which have become known as the Greaves and Kinlen hypotheses, are as follows:

- Greaves (9) hypothesized that many childhood leukemias, particularly common acute lymphoblastic leukemia (c-ALL) diagnosed in the childhood peak (2-5 years of age), arise as a consequence of a rare abnormal response to a common infection (Figure 2). Greaves stated that the infection may be of low pathogenicity and unusual in its timing and that genetic factors or susceptibility may influence the abnormal response leading to leukemia.
- Kinlen (23) proposed that leukemia (and possibly non-Hodgkin lymphoma) in children and young adults may be caused by a specific viral infection, the transmission of which is promoted by population-mixing. Drawing on animal models, he suggested that epidemics of the specific causative infection (and hence epidemics of leukemia) occur rarely and mostly in rural populations.

Although there is a large body of epidemiological data accruing on this topic, which is the subject of much current research, no viruses or definitive immunological mechanisms have yet been identified.

**Other childhood exposures.** Clearly, a number of other childhood exposures--aside from infections and immunological processes--are likely to influence adult health. The question for this review is whether the consequences of childhood exposure differ from the consequences of adult exposure. There is some suggestion that children may be more susceptible than adults to comparable doses of ionizing radiation and chemotherapy (108,123-125). Similarly, Truhan (126) suggested that exposure to excessive amounts of ultraviolet light in childhood may be the main cause of melanoma in adulthood.

The potential etiological role of diet in childhood also deserves particular mention. The influence of breastfeeding--which is clearly related to early life--is the subject of much current research. In this context it should be remembered that as well as the protective immunological effects postulated for conditions such as leukemia, deleterious effects--involving the transmission of agents such as HBV, human immunodeficiency virus, and fat-soluble chemicals--are also possible. Further, in these latter cases maternal exposure may have occurred several years before the index pregnancy (Figure 1).

## Animal Models

### Preconceptional/Transgenerational Effects

Transgenerational carcinogenesis, wherein the exposure of a parent before mating results in increased tumors in offspring and sometimes in subsequent generations, has been demonstrated for several types of radiation and a variety of chemical carcinogens [(17,127,128), Tables 6 and 7]. The tumors affected tend to be those that have a spontaneous incidence in the species and strain (lung, liver, or lymphoid system of mice) or are relatively easy to induce (skin and reproductive system in mice and nervous system in rats). Results for male-mediated transgenerational carcinogenesis are summarized in Table 6. Exposures of all possible stages of sperm development have given positive results--stem cells through mature sperm in the adult, and the embryonic gonocytes of gestation day (GD) 9 of mice and fetal testicular germ cells later in gestation in mice and hamsters.

Exposure	Parental agent	Exposure level	No. offspring	Effect
Male	1,2-Dichlorobenzene	100 mg/kg	100	Increased incidence of liver tumors

[illegible]

The weight of the evidence leaves little doubt that exposures of males can in fact lead to increased incidence of neoplasia in offspring. However, attempts to reproduce key findings have sometimes been partially or completely unsuccessful, indicating that the phenomenon is highly influenced by genetics, husbandry conditions, or other unknown variables. Thus, preconceptional carcinogenesis for the nervous system in rats by ethylnitrosourea (ENU) (129) could not be demonstrated with statistical significance in a later study (130). Preconceptional exposure to X rays of ICR or SHR (Swiss) mice led to increased lung tumors or increased susceptibility to postnatal induction of these tumors in offspring (131-133), but this effect could not be reproduced in BALB/c (134) or C3H (135) mice and only minimal effects were seen in CBA mice (136). X-irradiation of the spermatogonia of N5 mice resulted in increased incidence of leukemia in offspring (137); mice of this same strain were treated with X rays or tritium at the postmeiotic stage several decades later in another laboratory, and the treatment had a borderline effect on leukemia incidence (138).

Similarly, urethane had pronounced preconceptional carcinogenic effects on lung tumor incidence in ICR Swiss mice (131,132,139), but treatment of National Institutes of Health (NIH) Swiss male mice at the spermatid stage with this carcinogen had minimal effects on lung tumor incidence in offspring (140), and no significant effects in CBA mice (136). However, in the NIH Swiss mouse study, after paternal urethane treatment there were significant increases in incidences of pheochromocytomas in both sexes, lymphoma in females, and neoplasia of the forestomach in males (140); in CD-1 Swiss mice, urethane exposure of fathers led to significant increases in tumors of the liver in male offspring (141). To explain this wide variety of effects, the molecular mechanism of preconceptional carcinogenesis must be understood. When this is accomplished, more light may be shed on the likelihood of the phenomenon occurring in humans.

Only a few models have been investigated in sufficient detail to permit comparisons of the relative sensitivity of various stages of testicular development and spermatogenesis. In adult ICR mice, X-ray and urethane exposure appeared to have the greatest effect at the postmeiotic spermatid stage; spermatogonia were affected by X ray but not urethane (131). For ICR and LT mice, the effects in causing leukemia by paternal X-ray exposures were greatest for postmeiotic treatment, but for the sensitive N5 strain, spermatogonia were also affected (142). In C3H mice,  $^{252}\text{Cf}$  irradiation had a larger effect on liver tumors in offspring when given 14 days before mating, compared to 3 months before mating (143,144). Exposures of fetal male hamsters to *N*-nitrosodiethylamine (NDEA) on GDs 13 and 14, but not 12 or 15, caused significant increases in neoplasia in their offspring (145). Thus, special sensitivity of postmeiotic sperm, in which DNA repair does not occur, seems likely, but all other stages are also vulnerable, in theory, depending on the exposure agent and dose, genetic background, etc.

Less work has been carried out on preconceptional exposure of females (Table 7). Various intervals between 1 and 56 days before mating, encompassing mature oocytes, follicular stages, and primary oocytes, were comparably sensitive to effects of urethane, whereas effects of X rays were greatest between 8 and 21 days before mating, the postmeiotic stages, with earlier treatment leading to sterility (131). Mature oocytes, 1-7 days before mating, were vulnerable only to high acute doses of X ray; fractionated doses were without effect. Effects have also been noted after transplacental exposure of mouse fetuses to DES, with mid-gestation, late-gestation, and neonatal treatment having similar effects on the incidence of vaginal tumors in the F<sub>2</sub> descendants (146). Female hamster fetuses were less sensitive than their brothers to a multigenerational effect of transplacental NDEA, with a significant effect noted only after exposure on day 13 (145).

Species	Life history	Life span (years)	Sexual maturity	Reproduction
Atlantic salmon	Spawning in fresh water, migrating to sea	4-6	At sea	At sea
Atlantic cod	Spawning in sea	10-15	At sea	At sea
Atlantic herring	Spawning in sea	4-6	At sea	At sea
Atlantic mackerel	Spawning in sea	4-6	At sea	At sea
Atlantic plaice	Spawning in sea	4-6	At sea	At sea
Atlantic sole	Spawning in sea	4-6	At sea	At sea
Atlantic turbot	Spawning in sea	4-6	At sea	At sea
Atlantic halibut	Spawning in sea	4-6	At sea	At sea
Atlantic salmon	Spawning in fresh water, migrating to sea	4-6	At sea	At sea
Atlantic cod	Spawning in sea	10-15	At sea	At sea
Atlantic herring	Spawning in sea	4-6	At sea	At sea
Atlantic mackerel	Spawning in sea	4-6	At sea	At sea
Atlantic plaice	Spawning in sea	4-6	At sea	At sea
Atlantic sole	Spawning in sea	4-6	At sea	At sea
Atlantic turbot	Spawning in sea	4-6	At sea	At sea
Atlantic halibut	Spawning in sea	4-6	At sea	At sea

Walker and Kurth (147) carried out an interesting experiment on the mechanism of the multigenerational effect of DES in mice. Because the initial exposure was to the germ cell, the effect on the F<sub>2</sub> descendants could be mediated by an inherited genetic alteration, or indirectly by changes in the F<sub>1</sub> female developing from the germ cell (e.g., hormonal alterations). To test this, Walker and Kurth (147) transferred F<sub>2</sub> blastocysts among treated and control mothers. They found that either treatment of the blastocyst itself, or maternal treatment as a germ cell, was sufficient to cause an increase in uterine and ovarian tumors in the F<sub>2</sub> females as

adults. These effects appeared to be additive.

Thus, the rodent female germ cells, like those of the male, have theoretical sensitivity to preconceptional carcinogenic effects at every developmental stage, with actual effects dependent on agent, dose, and genetics, and with both direct and indirect effects potentially operative. The meaningfulness of these findings for human risk will not become clear until the mechanisms are understood. Although there is some evidence that preconceptional carcinogenesis involves conventional gene mutations showing Mendelian inheritance (131,132,137,139), in many contexts the high frequency of the effects and odd inheritance patterns suggest that other novel mechanisms are involved (148). Epigenetic alterations in control of gene expression, as in genetic imprinting, are a possibility (148); mutations in microsatellites (149) are another.

### Transplacental and Neonatal Carcinogenesis

Tables 8-21 summarize, by target tissue, published literature in which different stages of prenatal and/or neonatal development have been compared with regard to effects of chemical or radiation exposure within the same experiment. Each table also provides information regarding the ontogeny of that target tissue in rodents.

A number of factors have been suggested as determining susceptibility at different stages. These include a) numbers of target cells at risk, b) sensitivity to cell killing, c) effects of rate of cell division on fixation of mutation before repair can occur, d) ability to repair DNA damage, e) expansion of clones of mutated cells as part of normal ontogeny, f) presence of undifferentiated stem cells, g) development of differentiated characteristics, including the ability to carry out metabolic activation of chemicals, h) metabolic detoxification by placenta and/or maternal tissues, i) metabolic detoxification by the perinate itself, and j) immaturity of the endocrine and immunological systems. There is some experimental evidence for all of the above except for j, but this evidence is largely correlative in nature, and, albeit quite convincing in some situations, is in no case definitive. Several of these factors pertain concurrently. Rapid rate of cell division during organogenesis, the presence of numerous stem cells, the potential for generation of mutant clones, and limited placental development all characterize the middle third of gestation in the rodent, whereas the maximum number of cells at risk and the development of differentiated features are both features of the late fetus. Thus even strong correlative evidence must be interpreted with caution. It is also clear that likely factors are species, strain, tissue, and agent specific.

**Numbers of cells at risk.** Intuitively, it is clear that for a given dose of carcinogen and radiation, the likelihood of a mutational carcinogenic event will increase as a function of the number of target cells if all other factors are equal. This has been confirmed as likely for mouse skin throughout its development in the last half of gestation (Table 8), with X ray (150) or 7,12-dimethylbenz[a]anthracene (DMBA) (151,152) as the carcinogenic agents. Sensitivity was greatest at the end of gestation, when numbers of target cells are highest, and Morris et al. (152) further ruled out metabolic activation of DMBA as a likely contributing factor by studies of macromolecular adducts of the chemical.

Table 8. The ontogeny of the target tissue in the mouse embryo.

Organ	Developmental Stage	Approximate Cell Number	Approximate Cell Division Rate (per day)	Approximate Cell Survival Fraction	Approximate Cell Death Fraction
Brain	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Lung	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Spleen	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Testis	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Ovary	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Heart	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Stomach	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Intestine	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Bladder	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Uterus	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Vagina	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Prostate	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Adipose	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Muscle	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Skin	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Bone	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Cartilage	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Joint	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Eye	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Ear	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Nose	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Mouth	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Throat	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Trachea	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Esophagus	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Stomach	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Intestine	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Bladder	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Uterus	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Vagina	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Prostate	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Adipose	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Muscle	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Skin	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Bone	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Cartilage	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Joint	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Eye	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Ear	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Nose	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Mouth	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Throat	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Trachea	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Esophagus	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5

A similar result was obtained for mouse lung tumors caused by the direct-acting chemical ENU, but for a more limited developmental period (GDs 12-16), a period of exponential growth of undifferentiated pseudoglandular lung tissue (153). Peak ENU sensitivity of the mouse fetal lung on GD 16 has been demonstrated in many experiments (Table 9).

Table 9. The ontogeny of the target tissue in the mouse embryo.

Organ	Developmental Stage	Approximate Cell Number	Approximate Cell Division Rate (per day)	Approximate Cell Survival Fraction	Approximate Cell Death Fraction
Brain	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Lung	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Spleen	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Testis	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Ovary	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Heart	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Stomach	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Intestine	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Bladder	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Uterus	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Vagina	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Prostate	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Adipose	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Muscle	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Skin	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Bone	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Cartilage	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Joint	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Eye	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Ear	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Nose	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Mouth	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Throat	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Trachea	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5
Esophagus	GD 12-16	10 <sup>8</sup>	10 <sup>3</sup>	0.5	0.5

**Sensitivity of cells to killing.** During some stages of ontogeny, especially those with high rates of cell division, cells may be exquisitely sensitive to killing or growth suppression by genotoxic and other agents. It has been suggested that apparently low responsiveness to tumorigenesis during these times may indicate the destruction of sensitive cells, leading to a loss of target cells with neoplastic potential. Thus, cytotoxic radiation treatment before ENU exposure during late gestation of rats resulted in lower incidence of ENU-caused brain tumors (154). On an organism level, teratogenicity or embryonic death may be a more likely outcome than initiation of neoplasia during these stages. This has been studied systematically for urethane, for which mouse embryos that

survived the toxic effects did not develop tumors (155,156). Other chemicals and species have not been examined in detail for similar outcomes. Although a reduction in cell division after irradiation during organogenesis has been documented (157), an influence of this effect on tumor yield has not been demonstrated.

**Effects of rates of cell division.** Based on studies with cultured cells (158,159), sensitivity to mutation (and hence to initiation of neoplasms) can be directly proportional to mitosis rate because of increased frequency of fixation of mutation by replication before repair can occur. Recently, it was empirically demonstrated that the rate of mutation in the hamster embryo is maximum on GD 6, corresponding to the highest rate of cell division (160). The calculation of tumors induced per target cell per embryo or fetus for the rat, or per total cells for the hamster, confirmed that the risk per cell decreased as gestation progressed (161). The strongest correlative demonstration of this effect was provided for ENU initiation of lung tumors in fetal Swiss mice between GDs 15 and 19: numbers of adenomas induced were strongly correlated with numbers of cells in the cell cycle (162). However, the fact that the correlation was noticeably stronger for cells in G<sub>1</sub> compared to S or G<sub>2</sub> + M phases of the cell cycle suggests that factors in addition to DNA repair were important.

Influence of rate of cell division has also been suggested for the ovary, for which late embryos or middle-gestation mouse fetuses are more susceptible than those of later gestation to tumor initiation by urethane, ENU, DMBA, or azacytidine, but not those caused by radiation or a nitrosamine (Table 10). Interpretation of results for the ovary are complicated by the possibility that these are caused by indirect hormonally mediated effects (163).

Table 10. Tumor induction in the ovary of mice by various agents.					
Agent	Exposure schedule	Exposure	Target organ	Incidence	Reference
DMBA	GD 15-19	100 mg/kg	Ovary	100%	164
ENU	GD 15-19	100 mg/kg	Ovary	100%	165
5-Azacytidine	GD 15-19	100 mg/kg	Ovary	100%	166
Urethane	GD 15-19	100 mg/kg	Ovary	100%	167
Radiation	GD 15-19	100 rad	Ovary	0%	168
Nitrosamine	GD 15-19	100 mg/kg	Ovary	0%	169

The mouse embryo and early fetus were the most susceptible to causation of lymphocytic leukemias and lymphomas by DMBA, whereas GD 16 treatment with ENU or 5-azacytidine gave the highest yield of these malignancies (Table 11). However, the day 17 mouse fetus was not susceptible to causation of lymphoma or myeloid leukemia at doses of  $\gamma$ -rays effective in the neonate or adult (164,165). Findings for the nervous system and nitrosoureas, as related to the stage of greatest sensitivity, have also been mixed (Table 12).

Table 11. Tumor induction in the lymphatic system of mice by various agents.					
Agent	Exposure schedule	Exposure	Target organ	Incidence	Reference
DMBA	GD 15-19	100 mg/kg	Lymphatic system	100%	164
ENU	GD 15-19	100 mg/kg	Lymphatic system	100%	165
5-Azacytidine	GD 15-19	100 mg/kg	Lymphatic system	100%	166
Urethane	GD 15-19	100 mg/kg	Lymphatic system	100%	167
Radiation	GD 15-19	100 rad	Lymphatic system	0%	168
Nitrosamine	GD 15-19	100 mg/kg	Lymphatic system	0%	169

Table 12. Tumor induction in the nervous system of mice by various agents.					
Agent	Exposure schedule	Exposure	Target organ	Incidence	Reference
DMBA	GD 15-19	100 mg/kg	Nervous system	100%	164
ENU	GD 15-19	100 mg/kg	Nervous system	100%	165
5-Azacytidine	GD 15-19	100 mg/kg	Nervous system	100%	166
Urethane	GD 15-19	100 mg/kg	Nervous system	100%	167
Radiation	GD 15-19	100 rad	Nervous system	0%	168
Nitrosamine	GD 15-19	100 mg/kg	Nervous system	0%	169

**Ability to repair DNA damage.** This characteristic has been studied mainly for O-alkylated DNA bases, the principal mutagenic DNA lesions produced by many carcinogenic alkylating agents. O<sup>6</sup>-alkylguanine is a major promutagenic adduct resulting from the reaction of ENU or methyl nitrosourea (MNU) with DNA. Repair of this lesion by O<sup>6</sup>-alkylguanine-DNA alkyltransferase occurs at a considerably slower rate in the fetal rat brain, the target organ, than in other tissues (166-168). The level of the enzyme is maximum on GD 12, and decreases thereafter (169). However, this repair deficiency is similar in the adult (170), so it cannot account for the

50-fold greater sensitivity of the developing nervous system to tumor initiation by ENU. Similarly, this repair enzyme is lower in the brains of mice and gerbils than in liver and other tissues (171), but few neurogenic tumors are initiated in these species.

Repair in DNA strand breaks caused by methylmethanesulfonate was much less for fetal rat brain cells in culture compared to mouse brain and rat and mouse liver cells (172). The repair capacity for  $O^6$ -methylguanine was also very poor in fetal rat kidney compared to liver (168). Also in rats, DNA repair involving unscheduled DNA synthesis after MNU was more rapid in adults than in newborns and was not detected in fetuses (173). Repair of  $O^6$ -methylguanine was lower and levels of this adduct higher in newborn mice compared to adults given the same dose of *N*-nitrosodimethylamine (NDMA) (174). Human fetal cells in early passage showed differences in the extent of DNA excision repair after ultraviolet irradiation or ENU; skin and intestine were more active than liver, kidney, and brain (175).

However, certain other DNA repair enzymes are quite active in fetal tissues. Apurinic apyrimidinic endonuclease had expression throughout the rat fetus, with very high levels in fetal thymus, liver, and brain (176). The T:G mismatch-specific glycosylase was ubiquitously expressed in mouse fetuses up to GD 13.5, with high expression in the nervous system, thymus, lung, liver, kidney, and intestine by GD 14.5; at later stages it was prominent in thymus, brain, nasal epithelium, and proliferating regions of other tissues (177). The activity of 8-oxo-2'-deoxyguanosine 5'-triphosphate pyrophosphohydrolase, which eliminates oxidatively-damaged guanosine triphosphate, was higher in fetal mouse liver and lung than in the same tissues of the mothers (178).

In sum, comparative levels of DNA repair enzymes may influence the susceptibility of perinatal tissues to carcinogenesis, but definitive experimental evidence establishing this connection is still lacking.

**Expansion of clones of initiated cells.** After an embryonic or fetal cell sustains a mutation, the normal cell divisions of ontogeny may lead to expansion of the mutated (initiated) cell into a clone. This may reduce the latency of resulting tumors and increase the chances for further genetic changes needed for transformation of the initiated cells into a growing tumor. Also, the cells of the clone might be costimulatory for neoplastic development through paracrine factors. After transplacental ENU in mice, the lung and liver tumors initiated on day 10 are few in number because of the small target cell population but much larger and phenotypically unique compared to those initiated later (179). Similarly, lung tumors initiated by ENU on GD 15 were larger (162,180,181), more easily transplanted (180), and more papillary in phenotype (181) compared to those initiated later in gestation. This was also demonstrated for urethane-induced tumors (156), which were more likely to be of papillary type than those induced in their mothers.

**Presence of undifferentiated stem cells.** Evidence for a role for undifferentiated stem cells is strongest for fetal kidney (Table 13). The perinatal chemical induction in rats of nephroblastomas, which are similar to the Wilms' tumors of childhood, may require the presence of kidney stem cells. These tumors are not seen after transplacental exposure of mice, which establish a fully differentiated kidney by GD 17-18, or of hamsters, which have a functional kidney by GD 13. In the rat, on the other hand, stem cells are present until a few days after birth, and nephroblastomas are induced after exposure to ENU or MNU, with a maximum on GD 18 when fetal kidney reaches its peak size. However, some strains of rat (e.g., Noble) are more susceptible than others (F344 and Wistar), indicating the operation of additional genetic factors. In the opossum, organ development takes place mainly after birth, with kidney metanephric stem cells apparent for 6 weeks, and this tissue is sensitive to causation of nephroblastomas throughout this period (182). In rabbits, nephroblastomas are inducible by ENU, along with tubular cystadenomas, throughout the last 2 weeks of gestation (after a fully differentiated kidney is present). Information is lacking regarding the presence of stem cells in rabbit kidney during this time.

Table 13. Induction of nephroblastomas in various species by ENU and MNU.					
Species	Exposure period	Agent	Incidence	Notes	Reference
Rat	GD 17-18	ENU	100%	Maximum incidence at GD 18	183
Rat	GD 17-18	MNU	100%	Maximum incidence at GD 18	184
Mouse	GD 17-18	ENU	0%	Incidence 0% in fully differentiated kidney	185
Mouse	GD 17-18	MNU	0%	Incidence 0% in fully differentiated kidney	186
Hamster	GD 13	ENU	0%	Incidence 0% in fully differentiated kidney	187
Hamster	GD 13	MNU	0%	Incidence 0% in fully differentiated kidney	188
Opossum	Birth to 6 weeks	ENU	100%	Incidence 100% in undifferentiated stem cells	182
Rabbit	GD 25-28	ENU	100%	Incidence 100% in fully differentiated kidney	189
Rabbit	GD 25-28	MNU	100%	Incidence 100% in fully differentiated kidney	190

**Development of differentiated characteristics.** For some tissues and carcinogens, sensitivity increases markedly toward the end of gestation, as the tissue expresses more of its adult differentiation characteristics. This was studied systematically for hamster trachea, where tumorigenesis by NDEA correlated strongly with reduced rate of mitosis and presence of rough endoplasmic reticulum and secretion of mucopolysaccharide (183,184). Several other nitrosamines were also most effective in causation of laryngotracheal tumors on the last day of gestation in the hamster (185). Increased sensitivity at the end of gestation or in the newborn has been demonstrated for NDMA (186) and 1-phenyl-3,3-dimethyltriazene in rat kidney (187), NDMA and NDEA in mouse lung and liver (188-190), ENU and schwannomas and glioblastomas in C3H mice (189), several hydrazines and neurogenic tumors in rats (187), ENU and intraocular medulloepitheliomas in opossums (182), DBMA and forestomach and benign uterine tumors in mice (191), ENU and nephroblastomas in rabbits (192,193), and a variety of carcinogens in pituitary (Table 14) and forestomach tumors by DMBA (Table 15).

Table 14. Induction of pituitary tumors in various species by ENU and MNU.					
Species	Exposure period	Agent	Incidence	Notes	Reference
Rat	GD 17-18	ENU	100%	Maximum incidence at GD 18	194
Rat	GD 17-18	MNU	100%	Maximum incidence at GD 18	195
Mouse	GD 17-18	ENU	0%	Incidence 0% in fully differentiated pituitary	196
Mouse	GD 17-18	MNU	0%	Incidence 0% in fully differentiated pituitary	197
Hamster	GD 13	ENU	0%	Incidence 0% in fully differentiated pituitary	198
Hamster	GD 13	MNU	0%	Incidence 0% in fully differentiated pituitary	199

Chemical	Exposure	Species	Age	Sex	Response
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Harderian gland tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Harderian gland tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Renal mesenchymal tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Renal mesenchymal tumors

Some treatments are markedly more effective postnatally than transplacentally. These include urethane and ENU for causation of Harderian gland tumors in mice (Table 16); ENU and renal mesenchymal tumors in rats (Table 13); 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and lung and liver tumors in mice (Tables 9, 10, and 11); irradiation, urethane, and ENU and lymphoid tumors in mice (Table 11); radiation, ENU, benzidine, and safrole and liver tumors in mice (Table 17); 3'-azido-3'-deoxythymidine and mammary tumors in mice (Table 18); radiation and osteosarcoma in mice (Table 19), radiation and thyroid tumors in dogs (Table 20), and DES and uterine tumors in mice (Table 21). Certain types of tumors are more readily caused in adult than in neonatal animals (194), and certain chemicals are more effective in adult than in neonatal mice (195). Progressive acquisition of differentiated characteristics is among the several possible contributing factors, along with numbers of cells at risk and effective dose.

To skip tables 16-21 click [here](#).

Chemical	Exposure	Species	Age	Sex	Response
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Harderian gland tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Harderian gland tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Renal mesenchymal tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Renal mesenchymal tumors

Chemical	Exposure	Species	Age	Sex	Response
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ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Harderian gland tumors
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ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Renal mesenchymal tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Lymphoid tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Lymphoid tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Liver tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Liver tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Osteosarcoma
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Osteosarcoma
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Thyroid tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Thyroid tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Uterine tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Uterine tumors

Chemical	Exposure	Species	Age	Sex	Response
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Harderian gland tumors
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ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Lymphoid tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Liver tumors
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ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Uterine tumors

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ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Liver tumors
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ENU	0.05-0.5 mg/kg	Mice	1 day	Male	Uterine tumors
ENU	0.05-0.5 mg/kg	Mice	1 day	Female	Uterine tumors

A differentiated character that can influence susceptibility to perinatal carcinogenesis is the ability to activate chemically stable carcinogens to reactive DNA-damaging intermediates. This process is most often dependent on cytochrome P450s, which in rodents make their appearance during the last several days of gestation. In mice, metabolism of NDMA increased steadily in fetal livers from GD 16, where it was 3% of adult levels, through day 19 (13%), postnatal day 1 (25%) and day 4 (50%), reaching adult levels by day 7 (196). In human embryos, NDMA demethylase was detectable by 5-6 weeks after conception, and had increased 3-fold by 11-12 weeks (197). Also in mice, the genetic ability to respond to inducers of cytochrome P4501A1, which metabolizes polycyclic aromatic hydrocarbons, determined numbers of lung and liver tumors induced by 3-methylcholanthrene and DMBA [but, interestingly, not benzo(a)pyrene (BP)] (198-200), confirming that metabolic activation of carcinogens is a limiting factor in transplacental carcinogenesis for at least some chemicals.

For metabolism-independent oncogenic agents, such as ENU and radiation, it is obvious that factors other than cytochrome P450 levels must be operative if state of differentiation is in fact important. An influence of differentiation state, as opposed to total number

of target cells, seems most likely for tumor types inducible only during or after differentiation, as for intraocular medulloepitheliomas in opossums after ENU; Harderian gland tumors in mice after ENU; and myeloid leukemias in mice, mammary tumors in rats, and thyroid adenocarcinoma in dogs after irradiation. A change in the target cell within the organ also suggests a role for cellular differentiation. For example, ovarian tumors caused by ENU in mice were more likely to be tubular adenomas after prenatal exposure, but granulosa cell tumors after postnatal treatment (188). In rats, ENU caused nephroblastomas before birth but renal mesenchymal tumors after birth. Mechanistic information is not available for any of these situations.

**Maternal/placental metabolism.** Even if fetal tissues are unable to carry out metabolic activation of carcinogens, in certain cases a chemical may be activated by the maternal liver and the reactive intermediate delivered transplacentally to the fetus. The strongest case for such a situation was made in rats for procarbazine, which was adducted to DNA of fetuses but not to DNA of directly exposed neonates (201). After administration of BP to either pregnant mice (202) or pregnant patas monkeys (203), the levels of <sup>32</sup>P-postlabeled DNA adducts were similar in placenta and all fetal tissues, in spite of wide differences in the abilities of these tissues to activate BP, suggesting transplacental passage of maternally activated BP derivatives.

Although maternal metabolism can contribute to fetal risk in this way, detoxifying metabolism is in general more important. Mouse fetuses were more at risk of transplacental carcinogenesis by 3-methylcholanthrene or DMBA if their mothers were not responsive to induction of detoxifying metabolism of these compounds (198-200). Placental metabolism also contributes to detoxification: methylnitrosourea was an effective transplacental lung carcinogen in mice only on GD 9. This was related to the presence thereafter of effective hydrolase activity toward this compound in placenta, so that none reached the fetus after day 9 (204).

**Perinatal detoxification.** Urethane was 10-fold more effective at causing mouse lung tumors if administered < 12 hr before birth versus 24 hr before birth. Several investigations showed that this correlated perfectly with a 10-fold increase in total dose over time in the neonate, which cleared the urethane much more slowly than the pregnant mother (205,206). This important principle has not been studied for any other carcinogen but almost certainly pertains to most if not all that are chemically stable; most of the cytochrome P450 and the phase II detoxification enzymes (glucuronidases, epoxide hydrolases, etc.) are low in amount at birth, and reach adult levels only after 1-3 weeks. Poor capacity for metabolic detoxification is probably one of the major reasons for the high sensitivity of the neonate to chemical carcinogenesis.

**Endocrine or immunological control of neoplasia development.** Because endocrine and immunological systems are undeveloped in the perinate, it is possible that their absence permits the establishment of neoplasms that would be suppressed in adults. Although this idea is feasible, no concrete supporting evidence has been presented. A related concept is that perinatal exposures have long-term effects on the endocrine or immunological systems, which influence postnatal tumor development. This is an important possibility but has not been extensively explored. The exposure of fetal rats to DES led to an earlier appearance of mammary tumors caused by postnatal DMBA, and this effect was greater for DES given on GDs 10-13 compared to GDs 15-18 (207). This could have involved a direct effect of the DES on the mammary tissue, an indirect endocrine effect, or both. As noted previously, the multigenerational effect of DES could be mediated both directly by exposed blastocysts and by the maternal gestational environment (147). A number of plant-derived and anthropogenic compounds have endocrine-disrupting activity and may mimic many developmental effects of DES (208). Persistent endocrinological and immunological toxic effects of various perinatal exposures are well known; these should be studied systematically with regard to effects on postnatal carcinogenesis.

### Cross-Species Comparability

Can these principles from animal studies be extrapolated to humans? This would be most convincing for those observed in more than one animal species. Some of the factors that we described previously have been evidenced for only one species. Experiments implicating the presence of stem cells, levels of carcinogen-activating enzymes, and the presence of differentiated tissue have been reported for more than one species. Evidence for the induction of renal nephroblastomas from mice, hamsters, rats, rabbits, and opossums is in reasonable concurrence that renal stem cells must be present for this to happen. A role for fetal carcinogen metabolism has been directly demonstrated only for lung and liver in mice, but strong correlative evidence is available for kidney in rat and upper respiratory tract in hamsters. The apparent need for the presence of differentiated tissue for a neoplasm to be initiated has been seen in five species but with a different target tissue in each case. It is reasonable to believe that these factors are likely to be operative in the human, as well as others such as numbers of cells at risk, rates of cell division, and low capacity of the newborn for chemical detoxification. However, because of the high degree of species specificity in the target tissues affected, it is impossible to predict which tissue may be most vulnerable in humans.

The animal model most relevant to the human is the nonhuman primate, where structure of the placenta, length of gestation, and maturity of the fetus at birth resemble the human condition much more closely than is the case for rodents. In patas monkeys, transplacental ENU caused more tumors than the same dose given to juvenile monkeys, confirming the quantitatively higher sensitivity of the fetuses seen for this chemical in rodents (209). Tumor yield was similar for treatment throughout gestation and for more limited treatment during the first two-thirds of gestation, and few tumors resulted when treatment was not started until the beginning of the second trimester. These results suggested that sensitivity was highest during the first trimester. Neoplasms included relatively frequent vascular tumors and others of soft-tissue origin. There were also a few tumors comparable to human childhood cancers, including nephroblastoma, astrocytoma, glioma, oligodendroglioma, and leukemia, confirming the sensitivity of the primate fetus to chemical initiation of these neoplasms. Embryonal pulmonary blastomas were seen after transplacental exposure of rhesus but not patas monkeys, indicating species differences among primates.

### Animal Models of Human Childhood Cancers

Of the various animal tumors described here, only the nephroblastomas in rats, rabbits, opossums, and monkeys; osteosarcomas in mice; and lymphomas/leukemias in the mouse and dog have some direct relevance to common human childhood cancers. In

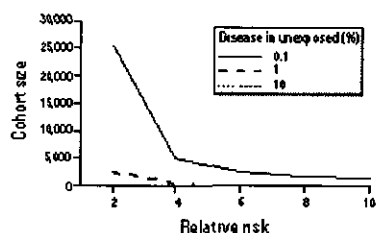
addition, cancers of the mammary gland and testis may occur in young people; these have been studied in relation to perinatal exposures in rats and mice. Nephroblastomas are a common outcome in rats, opossums, and rabbits after the exposure of well-developed kidneys that still retained a substantial proportion of stem cells. In all of these species (and in the nonhuman primate and in humans), nephroblastomas are apparently metanephric tumors resulting from a neoplastic deviation of the normal ontogeny of the metanephric blastema. This conclusion is supported by ultrastructural studies and by occasional findings of glomeruloid structures within Wilms' tumors. In the human embryo, the metanephros is present from the fifth through the seventh week of gestation. This suggests a narrow window of susceptibility if in fact some Wilms' tumors are caused by transplacental exposures.

More limited animal data for osteosarcoma, which results from radiation, suggest a broad window of sensitivity that is greatest in immature individuals. For lymphomas and leukemias, findings have been complex. Myeloid leukemias have been reported only after postnatal radiation exposure. Lymphomas and leukemias have been increased after perinatal exposure in some studies, with the time of highest sensitivity possibly depending on the oncogenic agent. This time was late in gestation or after birth for ENU,  $\gamma$ -radiation, urethane, and X rays but early in gestation for DMBA. Treatments with nucleoside analogues led to decreases in hematopoietic neoplasms. In the experiment of Schmahl et al. (210), 5-azacytidine caused either an increase or a decrease in these cancers, depending on the exact dose and GD of treatment. These findings point to the possibility of perinatal windows of exposure sensitivity to the induction of lymphomas and leukemias in humans but fail to give more specific useful information.

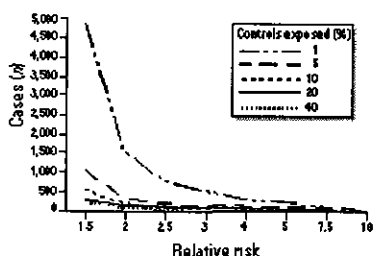
## Gaps in Knowledge

### Humans

Although animal models have shown that cancer risk can be increased after exposure to certain potentially hazardous agents—preconceptionally, *in utero*, and perinatally—in humans, much of the evidence is equivocal. This is partly because findings from studies that have investigated the etiology of cancer in relation to prenatal/early life exposures are often based on small numbers of cases because both cancer in young people and many of the potentially hazardous exposures studied are rare. Figures 3 and 4 compare the statistical power of cohort studies (disease onset in exposed individuals compared to disease onset in unexposed individuals) and case-control studies (exposures in individuals with the disease compared to exposures in individuals without the disease) to detect differences, should they exist. Figures 3 and 4 show the number of subjects required to give an 80% chance of detecting increased risks at different exposure levels for typical study designs. Exposure levels below a general population level of 0.1% are not uncommon in epidemiological studies [paternal occupational exposure to ionizing radiation at work, for example, would fall into this category (Tables 3 and 4)]. For a rare exposure such as this, to have an 80% chance of detecting a trebling in risk, disease rates in two cohorts of equal size of approximately 15,000 subjects would have to be monitored over time (Figure 3) or past exposures in approximately 600 cases and 1,200 controls would have to be compared (Figure 4). Unfortunately, studies as large as these are rarely conducted and many results are based on insufficient data.



**Figure 3.** Exposed cohort size needed to have an 80% chance of detecting a difference ( $p < 0.05$ , two-sided)—equal size comparison group.



**Figure 4.** Cases necessary to have an 80% chance of detecting a difference ( $p < 0.05$ , two-sided). Case-control study with a 1:2 ratio.

Most epidemiological studies that have investigated the etiology of cancer in young people have had a case-control design. Although valid diagnostic information on the malignancy in question is generally collected, good quality information on the exposure(s) is not always available. This is a particular problem for studies investigating potential etiological factors relating to lifestyle—such as diet and smoking—where data are often self-reported. When information about exposure is only obtained for those who participate in a study, response/recall bias can be exacerbated by participation bias, which is introduced when those who respond to a study differ from those who do not. Because participation rates are often lower among controls than among cases, the potential for producing false negative and false positive findings in case-control studies relying on self-reported exposure information is problematic.

An additional difficulty in human studies is that even when exposure data are good, pinpointing times of exposure and quantifying

dose is rarely straightforward. For example, pesticide exposure of mothers at work could affect their germ cells, their fetuses, or their children after birth. In some cases, exposure may have occurred in only one time period (such as neonatal administration of vitamin K or immunization), but for others it may be impossible to determine the relevant exposure period (such as maternal smoking). Individual exposures and the timing of exposure cannot always be readily isolated one from another and observed associations with one factor may simply reflect associations with others.

Because epidemiological studies are not conducted in laboratories, specific questions about critical time windows in relation to cancer are not easily answered. This is true for exposures at all stages of life, not just prenatally and in childhood. With respect to specific exposures, the situation is much as it has been for the past 20 years. For the preconceptional period, no exposures are accepted as definitively associated with preconceptional carcinogenesis. For the *in utero* period, only ionizing radiation and DES are agreed, and even for these, there is still debate about timing and dose. However, although no responsible exposure has been identified, the molecular evidence of Ford et al. (15,16) linking genetic changes initiated *in utero* to subsequent leukemia development has given added impetus to research in this area. For perinatal/postnatal exposures the situation is perhaps less bleak, particularly for those exposures that are only relevant postnatally. With respect to critical time windows of exposure, the etiological role of infectious disease in cancer development is one of the most active areas of current research (9,121). Developments in this area, like those described by Ford et al. (15,16), require collaboration between epidemiologists and laboratory-based scientists. Indeed, the use of molecular techniques in epidemiology has increased markedly over the last few years; perhaps it is through collaborations such as these that questions about the importance of exposure dose and timing in cancer etiology will finally be resolved.

### Animal Models

Notable gaps in the rodent data are of two types: mechanistic information about the reasons for observed periods of high sensitivity and tests of both risk situations and concepts arising from the human data. In the first category, there are presently more gaps than firmly woven stories. None of the likely susceptibility factors have been investigated thoroughly enough for the information to be used with assurance in the human context. For some of the factors, this could easily be done by the use of several carcinogenic agents and species and with carefully planned investigations. For others, such as DNA repair, more basic information is needed. Putative involvement of particular enzymes or cell-control molecules could be tested with current technology for selective gene inactivation and targeted overexpression by transgenes. For example, one could test whether transplacental carcinogenesis by certain nitrosamines in mice is dependent on activation by cytochrome P4502E1 by using the already available mice knocked-out in this gene. Differential gene expression techniques could be used to search for those genes whose increase or decrease in expression corresponds to changes in sensitivity to tumor initiation.

With regard to specific cross-referencing to human studies, much more work could be done on risk factors of special concern. For example, preconceptional carcinogenicity of radiation has been studied with only a few animal models, and with a highly variable outcome, apparently depending on the dose and type of radiation and the species and strain of test animal. This issue could be systematically investigated. Paternal tobacco smoking has been implicated in several studies, but few of the carcinogens present in tobacco smoke have been tested as preconceptional carcinogens, and none have been specifically tested in males. Furthermore, available data from animal models hint at a novel mechanism in play. When eventually uncovered, this knowledge of mechanism could help in understanding human risk from paternal radiation exposure, tobacco smoking, etc. Recently suspected human risk factors such as vitamin K and topoisomerase II inhibitors have not been tested in perinatal animal models. Although neonatal rodents are more susceptible to infections than adults, including infection by oncogenic viruses, they have not yet been used to model the cancer-risk role proposed for human infant exposure to microbes.

There is increasing knowledge of the molecular and genetic changes in human childhood cancers. The meaningfulness of the comparable animal model neoplasm as a risk indicator would be indicated by similarities or dissimilarities in molecular etiology. For example, several tumor-susceptibility genes or chromosomal areas have been identified in human Wilms' tumors of the kidney; these could be examined in rat and rabbit nephroblastoma. Similarly, K-ras oncogene mutations have been found in rat nephroblastomas but apparently have not been tested for Wilms' tumors.

It should be emphasized that at present it seems highly unlikely that any of this work will ever be done. Only a handful of laboratories worldwide are working on perinatal carcinogenesis in animal models. If regulatory agencies desire these data, they will have to demand (and pay for) them. Large, long, expensive studies will be needed. Industry might be required to do certain bioassays but cannot be expected to gather much of the bioassay or any of the mechanistic information.

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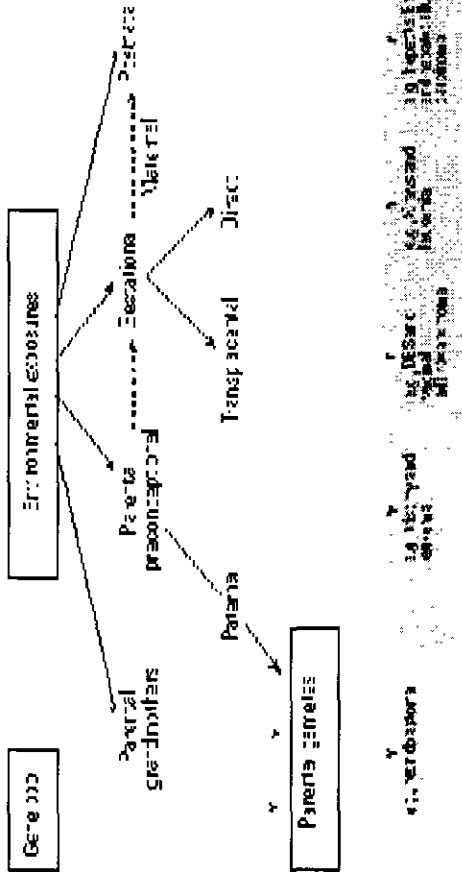


Table 1. Number of cases (%) and sex ratios (male/female) by age for England and Wales, 1951-1990

Cancer	Total (%)	Age (years)				
		0	1-4	5-9	10-14	15-19
Leukemia	3,235 (33)	38	12	12	14	12
Lymphoid	2,988 (38)	37	12	13	16	12
Myeloid	1,341 (40)	DE	19	3E	21	25
Prostate	491 (4)	-	38	34	19	23
Testis	581 (6)	33	16	39	24	25
Bladder	2,845 (39)	10	12	12	13	12
Brain and spinal	2,732 (4)	1E	13	1E	11	15
Esophagus	907 (9)	38	12	10	12	11
Stomach	797 (7)	10	11	13	13	11
Rectum	312 (3)	14	10	13	-	12
Colon	664 (6)	11	9	DE	11	0.3
Small intestine	101 (1)	11	25	37	12	14
Stomach	541 (5)	-	0.8	DE	10	0.3
Colon	294 (3)	-	16	DE	10	0.3
Soft tissue sarcoma	771 (7)	13	13	11	11	12
Endometrial	337 (4)	20	13	DE	18	0.3
Ovaries	341 (3)	-	0.8	10	18	0.5
Uterus	1,382 (10)	11	12	13	13	12

DE = data not available

Table 2. Data for 4 2000 mg/kg bw/day oral dose studies in rats, showing the effect of dose on the response to the 4 2000 mg/kg bw/day oral dose studies in rats.

Dose	Total weight				Weight				Body weight			
	1	2	3	4	1	2	3	4	1	2	3	4
1000	10	10	10	10	10	10	10	10	10	10	10	10
2000	10	10	10	10	10	10	10	10	10	10	10	10
3000	10	10	10	10	10	10	10	10	10	10	10	10
4000	10	10	10	10	10	10	10	10	10	10	10	10
5000	10	10	10	10	10	10	10	10	10	10	10	10
6000	10	10	10	10	10	10	10	10	10	10	10	10
7000	10	10	10	10	10	10	10	10	10	10	10	10
8000	10	10	10	10	10	10	10	10	10	10	10	10
9000	10	10	10	10	10	10	10	10	10	10	10	10
10000	10	10	10	10	10	10	10	10	10	10	10	10
11000	10	10	10	10	10	10	10	10	10	10	10	10
12000	10	10	10	10	10	10	10	10	10	10	10	10
13000	10	10	10	10	10	10	10	10	10	10	10	10
14000	10	10	10	10	10	10	10	10	10	10	10	10
15000	10	10	10	10	10	10	10	10	10	10	10	10
16000	10	10	10	10	10	10	10	10	10	10	10	10
17000	10	10	10	10	10	10	10	10	10	10	10	10
18000	10	10	10	10	10	10	10	10	10	10	10	10
19000	10	10	10	10	10	10	10	10	10	10	10	10
20000	10	10	10	10	10	10	10	10	10	10	10	10
21000	10	10	10	10	10	10	10	10	10	10	10	10
22000	10	10	10	10	10	10	10	10	10	10	10	10
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28000	10	10	10	10	10	10	10	10	10	10	10	10
29000	10	10	10	10	10	10	10	10	10	10	10	10
30000	10	10	10	10	10	10	10	10	10	10	10	10
31000	10	10	10	10	10	10	10	10	10	10	10	10
32000	10	10	10	10	10	10	10	10	10	10	10	10
33000	10	10	10	10	10	10	10	10	10	10	10	10
34000	10	10	10	10	10	10	10	10	10	10	10	10
35000	10	10	10	10	10	10	10	10	10	10	10	10
36000	10	10	10	10	10	10	10	10	10	10	10	10
37000	10	10	10	10	10	10	10	10	10	10	10	10
38000	10	10	10	10	10	10	10	10	10	10	10	10
39000	10	10	10	10	10	10	10	10	10	10	10	10
40000	10	10	10	10	10	10	10	10	10	10	10	10
41000	10	10	10	10	10	10	10	10	10	10	10	10
42000	10	10	10	10	10	10	10	10	10	10	10	10
43000	10	10	10	10	10	10	10	10	10	10	10	10
44000	10	10	10	10	10	10	10	10	10	10	10	10
45000	10	10	10	10	10	10	10	10	10	10	10	10
46000	10	10	10	10	10	10	10	10	10	10	10	10
47000	10	10	10	10	10	10	10	10	10	10	10	10
48000	10	10	10	10	10	10	10	10	10	10	10	10
49000	10	10	10	10	10	10	10	10	10	10	10	10
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51000	10	10	10	10	10	10	10	10	10	10	10	10
52000	10	10	10	10	10	10	10	10	10	10	10	10
53000	10	10	10	10	10	10	10	10	10	10	10	10
54000	10	10	10	10	10	10	10	10	10	10	10	10
55000	10	10	10	10	10	10	10	10	10	10	10	10
56000	10	10	10	10	10	10	10	10	10	10	10	10
57000	10	10	10	10	10	10	10	10	10	10	10	10
58000	10	10	10	10	10	10	10	10	10	10	10	10
59000	10	10	10	10	10	10	10	10	10	10	10	10
60000	10	10	10	10	10	10	10	10	10	10	10	10
61000	10	10	10	10	10	10	10	10	10	10	10	10
62000	10	10	10	10	10	10	10	10	10	10	10	10
63000	10	10	10	10	10	10	10	10	10	10	10	10
64000	10	10	10	10	10	10	10	10	10	10	10	10
65000	10	10	10	10	10	10	10	10	10	10	10	10
66000	10	10	10	10	10	10	10	10	10	10	10	10
67000	10	10	10	10	10	10	10	10	10	10	10	10
68000	10	10	10	10	10	10	10	10	10	10	10	10
69000	10	10	10	10	10	10	10	10	10	10	10	10
70000	10	10	10	10	10	10	10	10	10	10	10	10
71000	10	10	10	10	10	10	10	10	10	10	10	10
72000	10	10	10	10	10	10	10	10	10	10	10	10
73000	10	10	10	10	10	10	10	10	10	10	10	10
74000	10	10	10	10	10	10	10	10	10	10	10	10
75000	10	10	10	10	10	10	10	10	10	10	10	10
76000	10	10	10	10	10	10	10	10	10	10	10	10
77000	10	10	10	10	10	10	10	10	10	10	10	10
78000	10	10	10	10	10	10	10	10	10	10	10	10
79000	10	10	10	10	10	10	10	10	10	10	10	10
80000	10	10	10	10	10	10	10	10	10	10	10	10
81000	10	10	10	10	10	10	10	10	10	10	10	10
82000	10	10	10	10	10	10	10	10	10	10	10	10
83000	10	10	10	10	10	10	10	10	10	10	10	10
84000	10	10	10	10	10	10	10	10	10	10	10	10
85000	10	10	10	10	10	10	10	10	10	10	10	10
86000	10	10	10	10	10	10	10	10	10	10	10	10
87000	10	10	10	10	10	10	10	10	10	10	10	10
88000	10	10	10	10	10	10	10	10	10	10	10	10
89000	10	10	10	10	10	10	10	10	10	10	10	10
90000	10	10	10	10	10	10	10	10	10	10	10	10
91000	10	10	10	10	10	10	10	10	10	10	10	10
92000	10	10	10	10	10	10	10	10	10	10	10	10
93000	10	10	10	10	10	10	10	10	10	10	10	10
94000	10	10	10	10	10	10	10	10	10	10	10	10
95000	10	10	10	10	10	10	10	10	10	10	10	10
96000	10	10	10	10	10	10	10	10	10	10	10	10
97000	10	10	10	10	10	10	10	10	10	10	10	10
98000	10	10	10	10	10	10	10	10	10	10	10	10
99000	10	10	10	10	10	10	10	10	10	10	10	10
100000	10	10	10	10	10	10	10	10	10	10	10	10

Standard error of the mean is shown in parentheses.



Table 4. Subsequent analysis due to the inclusion of the interaction term between the type of treatment and the type of outcome variable.

Polymer	Groups	C <sub>60</sub> analog		Total	C <sub>60</sub> analog % C <sub>60</sub>
		Wt. %	mol. %		
Orderies (99-83) 27.4	adhered to H <sub>2</sub>	1	15	25	3
	adhered	5	15	40	3
Unreacted (187) 0.3	adhered to H <sub>2</sub>	18	12	40	1
Pure co. (305) 0.1	adhered to H <sub>2</sub>	5	3	7	10
Midnight (117) 20.70	adhered	10	12	7	10
Duponts (305) 1.1	U re groups	10	10	21.70	70
	adhered to H <sub>2</sub>	10	10	40.30	30
	U re groups	10	10	21.70	70
	adhered to H <sub>2</sub>	10	10	40.30	30
Pure co. (305) 0.1	adhered to H <sub>2</sub>	10	10	40.30	30
	adhered	10	10	40.30	30
	U re groups	10	10	40.30	30
	adhered to H <sub>2</sub>	10	10	40.30	30

1. The first step is to identify the problem. In this case, the problem is that the company is not meeting its sales targets.